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13. ABSTRACT (Maximum 200 Words) One of the earliest common changes in the development of breast cancer occurs when some breast epithelial cells begin to grow and proliferate independently of estradiol (E2). We have conducted studies on model breast epithelial cell lines: MCF7 cells, whose proliferation is dependent on E2; and LCC1 cells, a cell line derived from MCF7 cells with an acquired E2 independence for growth. We have continued to apply proteomics techniques (two-dimensional electrophoresis, image analysis and protein identification by mass spectrometry) to characterize broadly the patterns of protein expression in these two cell lines and their regulation by E2. We have identified several dozens of proteins in MCF7 cells which are up- or down-regulated in association with E2-controlled proliferation. Many of these are seen constitutively altered in the LCC1 cells grown in the absence of E2. While there is little or no effect of E2 in the proliferation of LCC1 cells, we find many proteins whose levels /are/ altered by the addition of E2. Our results are consistent with the hypothesis the E2-dependent MCF7 cells undergo apoptosis upon removal of E2 while the LCC1 cells have lost the ability to induce apoptosis upon removal of E2 and thereby display E2-independent growth.				
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INTRODUCTION

One of the earliest common changes in the development of breast cancer occurs when some breast epithelial cells begin to grow and proliferate independently of estradiol (E2). We have been conducting studies on model breast epithelial cell lines: MCF7 cells, whose proliferation is dependent on estradiol; and LCC1 cells, a cell line derived from MCF7 with an acquired E2 independence for growth. We continued to apply proteomics techniques (two dimensional electrophoresis, image analysis and protein identification by mass spectrometry) to characterize broadly the patterns of protein expression in these two cell lines and their regulation by estradiol. We have identified several dozens of proteins in MCF7 cells which are up- or down-regulated in association with E2-controlled proliferation. Many of these are seen constitutively altered in the LCC1 cells grown in the absence of E2. While there is little or no effect of E2 on the proliferation of LCC1 cells, we find many proteins whose levels are altered by the addition of E2. Our results are consistent with the hypothesis that E2-dependent MCF7 cells undergo apoptosis upon removal of E2 while the LCC1 cells have lost the ability to induce apoptosis upon removal of E2 and thereby display E2-independent growth.

BODY

Representative silver-stained 2D gels of total proteins isolated from MCF7 cells minus 17- β -estradiol (E2) covering three pH ranges are shown in Figure 1. Overlapping pH ranges are used to increase the number of considered protein features for a given complex sample. These images represent three of the approximately 500 such gels that have been through the course of this work. We have had good reproducibility in the gel images obtained for total cell proteins isolated from the various cell conditions tested: MCF7 cells plus or minus E2 and LCC1 cells plus or minus E2. Numerous attempts at subcellular fractionation and membrane isolation, while largely successful as judged by microscopy and 2D-gel based proteome analysis, were not sufficiently reproducible to allow precise analysis of differential protein expression.

Most of the initial time in this study was spent replicating Dr. Clarke's conditions as closely as possible by harvesting the cells 24 hours after addition of E2 to the medium. The effect of E2 on the proteomes of the MCF7 cells was virtually non-existent. That is to say the proteins detected in 2D gels from estrogen-stimulated cells were virtually indistinguishable from those of unstimulated cells. More consistent and numerous changes were observed when comparing MCF7 with LCC1 cells. As mentioned in the previous report (May 2001) we find a pronounced 1 day lag between the time of E2 addition and detection of increased growth of MCF7 cells. All studies in the present report were after re-stimulation of the cells with E2 for 48 hours whereby we observe many more significant and reproducible proteome changes (summarized below).

The switch from analyzing our gels using the Phoretix 2D gel analysis software (Nonlinear Dynamics) to using the more advanced Progenesis gel analysis software from the same vendor to take advantage of the enhanced spot detection, image warping, and database features of this new software. Even so, with our silver stained images, considerable time and effort was still required for spot editing and matching. We have completed the analysis of the pH 5-8 gels and have nearly completed the analysis of the pH 4-7, and 7-10 gel images. Spot detection is illustrated in Figure 2 and image warping and spot matching is illustrated in Figure 3. We use averaged gels constructed from multiple individual gels for a given condition. For a protein feature to be

considered it must appear and be matched in at least 4 out of 6 individual gels. For pH 5-8 gels, total number of spots satisfying this criterion for each condition are listed in Table 1. Table 2 summarizes the total protein changes observed upon addition of E2 to MCF7 and LCC1 cells and the effect of acquired E2 independent growth. Table 3 shows that there are very few E2-induced changes held in common between MCF7 and LCC1 cells whereas there are many changes held in common between the effect of E2 on MCF7 cells and the effect of acquired E2 independent growth (Table 4). The 73 proteins which increase (Tables 2 and 4) as a result of acquired E2 independent growth of LCC1 cells are potential markers for early detection of breast cancer. Preliminary analysis of the pH 7-10 gels shows comparatively few changes in protein expression induced by E2 or by the phenotype switch. As expected, analysis of the pH 4-7 gels confirms many of the changes summarized in Table 2-4 and add a few more acidic proteins. Summary statistics of the overlap and additional protein coverage afforded by these other pH ranges is not yet completed.

We have now identified most of the hundreds of protein alluded to in tables 2 through 4 by a combination of MALDI TOF MS peptide mass mapping and LC ESI MSMS analysis. The most consistent pattern emerging is a strong correlation with cell proliferation of proteins associated with polynucleotide synthesis and processing, protein synthesis, and nuclear transport. This is not surprising. There are also several changes observed in post-translationally modified protein species of nuclear lamins, several cytokeratins, and some chaperonins. One of the more interesting findings is that when E2 is removed from MCF7 cells and the cells cease to proliferate or even decrease in number, we find specific truncated forms of cytokeratins 18 and 19 appear (for example see Figures 4, 5 and 6). The full length cytokeratins 18 and 19 (not labeled) are found in the center left region of the gel (Figure 4) among the dark cluster of abundant poorly resolved proteins. Detailed sequence analysis using the mass spectrometry data suggests that all of these specific truncated forms are consistent with the action of pro-apoptotic caspases.

We examined further the idea that acquired estradiol independent growth in LCC1 cells results from a loss of activation of apoptosis upon removal of estradiol. The results of cell shakeoff and reattachment assays we have done are consistent with induction of apoptosis by removal of E2 from MCF7 but not LCC1 cells. More accurate flow cytometry experiments to determine the proportions of cells in G2/M phase, G1/G0, and cells with sub-G0 DNA content (indicative of apoptotic cells) for each of our four conditions (Figure 7). These results support the notion that E2-dependent MCF7 cells appear to leave S/G2/M phase and a fraction of them undergo apoptosis (as evidenced by the sub-G0 DNA content) upon removal of E2. The E2-independent LCC1 cells show a much smaller effect of removing E2 from the growth medium. To ask what pathways might be involved in regulating apoptosis we asked whether there were any changes in PARP cleavage products, indicators of induced cell death. We also asked whether apoptosis is constitutively suppressed in LCC1 cells as opposed to not being so in MCF7 cells by looking at the phosphorylation status of Akt1. Many cell survival signals work by suppressing apoptosis through this pathway. The results of these experiments were inconclusive since we failed to find experimental conditions that gave good reproducible results by Western blotting techniques.

Complete summary statistics on protein changes in these experiments remain to be compiled for the manuscript being written for publication.

KEY RESEARCH ACCOMPLISHMENTS

Task 1. Set up 2D-electrophoresis system to analyze and compare the proteomes of MCF7 and MCF7/LCC1 breast cells and prepare initial 2D-gels for mass spectrometry protein identification. (*month 1-3*)

Completed in Year 1

Task 2. Implement software and techniques for producing a master gel pattern whereby changes in protein expression patterns among breast cell lines can be recognized with computer assistance. (*months 1-8*)

Completed in Year 1 and improved in year 2 (see body of report)

Task 3. Use mass spectrometry to sequence and identify remaining members of the set of abundant proteins that reflect the MCF7 response to estrogen and the MCF7/LCC1 acquisition of estrogen independence. (*months 2-4*)

Completed in year 2.

Task 4. Further elucidate the differences between MCF7 and MCF7/LCC1 cells and the response of these cells to estrogen to find those proteins that have eluded detection by virtue of their lower abundance (*months 6-18*) and/or previously unexplored isoelectric point range. (*months 9-21*)

Begun in year 1, extensive data collected in year 2, data collection completed in year 3, analysis is nearly complete..

Task 5. We will perform subcellular fractionation to characterize the proteomes of nuclear, soluble, and especially the membrane fractions. (*months 12-24*)

Begun in year 1, completed in year 2. This approach was judged too irreproducible for meaningful comparison of different cells grown under various conditions. The data are useful, however, for indicating which subcellular compartment a particular protein feature is associated with.

Task 6. Rapidly evolving improvements in mass spectrometry technology and database searching software will be implemented to improve sensitivity and to better sequence and identify newly selected proteins of lower abundance. (*months 1-24*)

We have continued to improve our general laboratory material handling practices allowing greater throughput and sensitivity in our mass spec analyses of protein features identified in gels. We have implemented a much more powerful image analysis package (Progenesis, Non-linear dynamics) than we were previously using. A new research grade MALDI TOF mass spectrometer (Reflex IV, Bruker Daltonics) was installed in year 2 allowing much more sensitive and accurate peptide mass mapping for protein identification. We have acquired and installed MASCOT (Matrix Science) for doing automated peptide mass map sequence database

searching on a local server. This has allowed higher throughput than using web-based search engines. MASCOT also provides a complement to our SEQUEST database searches of LC-MSMS data.

REPORTABLE OUTCOMES

Steven H. Seeholzer, Anthony T. Yeung, Bryan D. Oconnell, and Robert C. Clarke (2002). Proteomic analysis of Estradiol Independent Growth of a MCF7 Derivative Cell Line. *Era of Hope: Department of Defense Breast Cancer Research Program Meeting*, Orlando, Florida.

We shall presently finish the above reported work and prepare a manuscript for publication in a refereed journal. Remaining details to accomplish this task are to integrate the analyses of the overlapping pH range gels with each other, prepare final figures, and confirm some of the more tentative protein identifications by mass spectrometry.

CONCLUSIONS

We have confirmed many of the expected E2-induced protein changes in MCF7 and LCC1 cells and have extended these observations to many newly identified protein markers associated with acquired estradiol independent growth. Estradiol appears to suppress apoptosis in dependent MCF7 cells whereas apoptosis appears to be constitutively suppressed in the estradiol-independent LCC1 cells.

REFERENCES

Skaar TC. Prasad SC. Sharareh S. Lippman ME. Brunner N. Clarke R. Two-dimensional gel electrophoresis analyses identify nucleophosmin as an estrogen regulated protein associated with acquired estrogen-independence in human breast cancer cells. *Journal of Steroid Biochemistry & Molecular Biology*. 67(5-6):391-402, 1998

Figures and Tables.

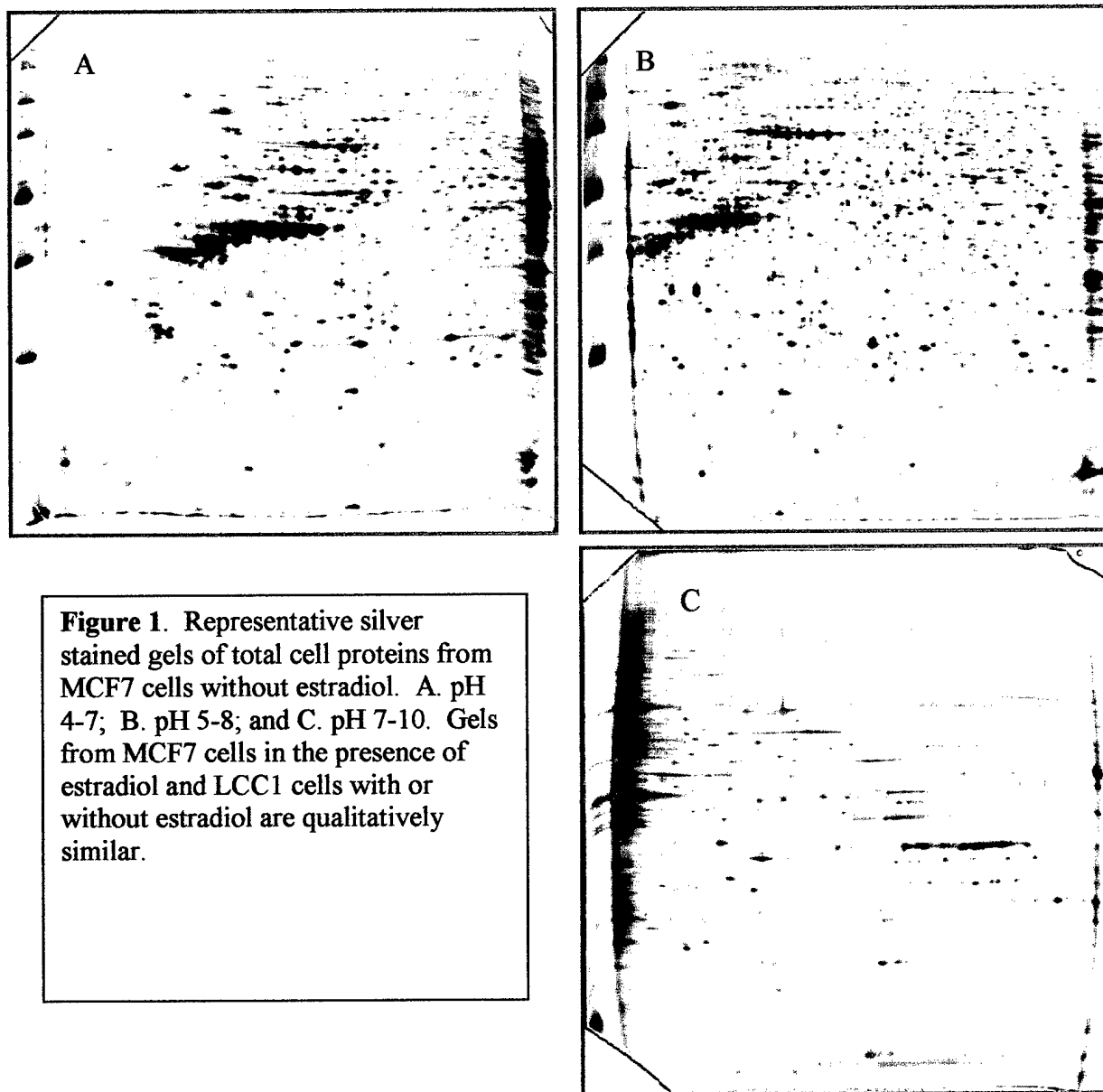


Figure 2. Illustration of spot detection by Progenesis software. Extensive editing of spot detection was found to be necessary in order to rejoin inappropriately split spots and split inappropriately joined spots.

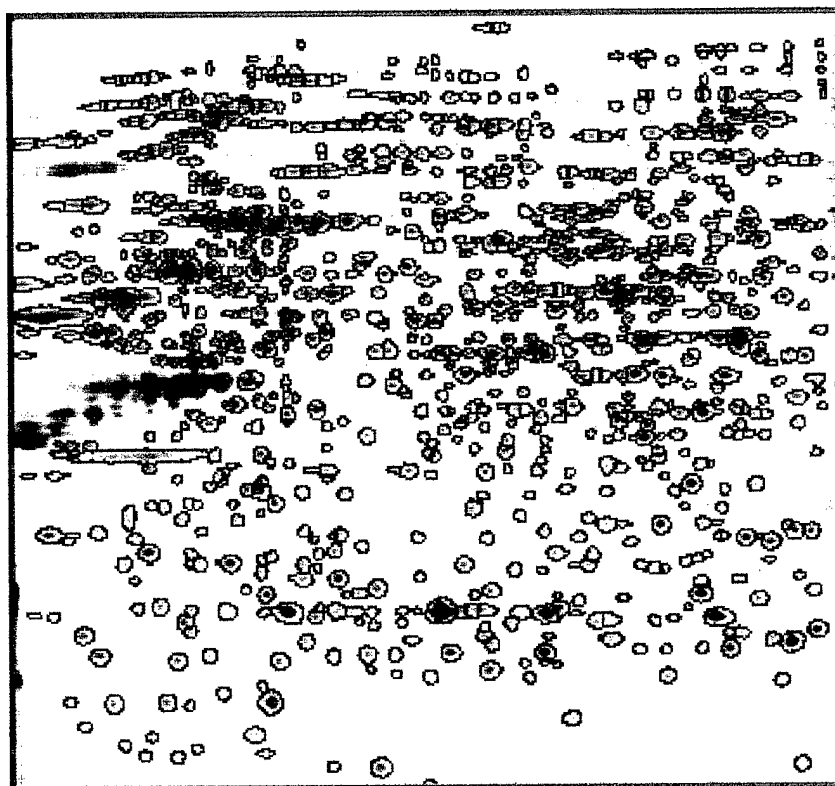
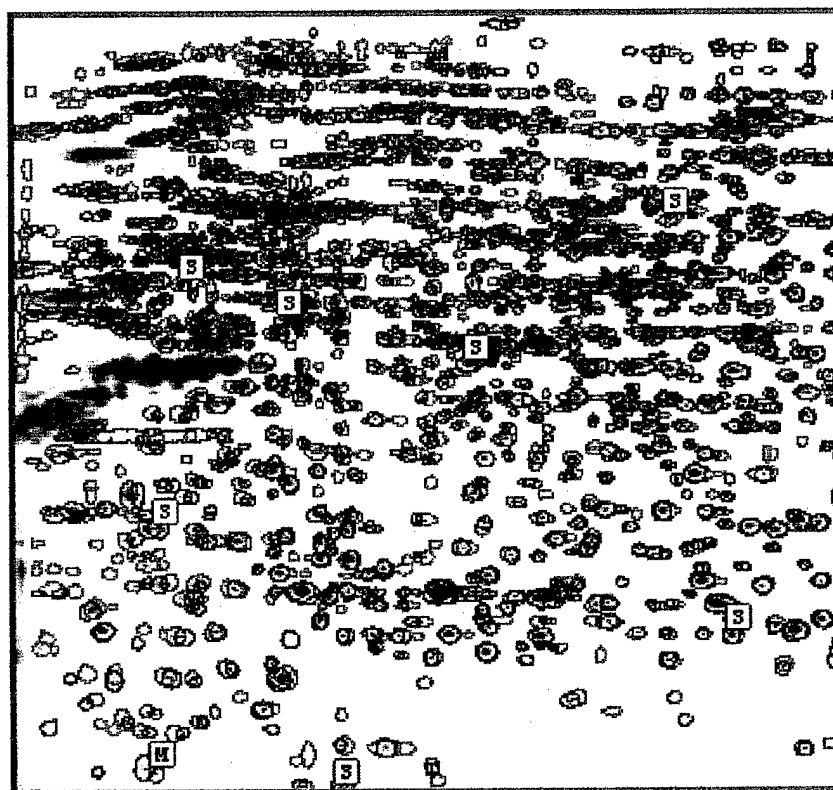


Figure 3. Illustration of gel image warping and spot matching procedure. User selected seeds for matching were chosen from highly reproducible local constellations of protein features. User seeds were invariably shown by mass spectrometry to be the same protein.



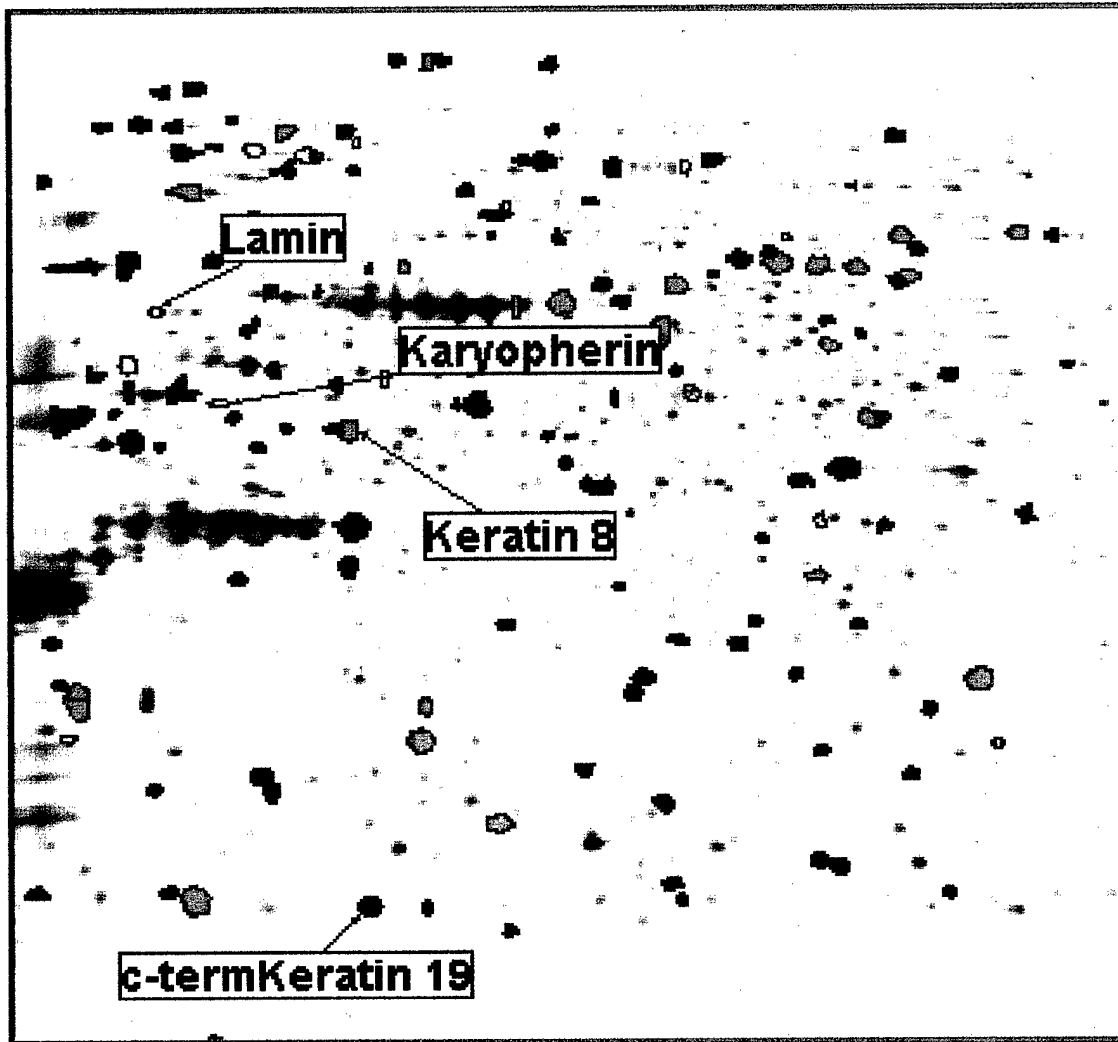


Figure 4. Gel image of MCF7 -E2 with E2-induced changes in MCF7 cells superimposed in color. Blue spots represent protein features not found in MCF7 +E2 but found in MCF7 -E2 (eg. C-term fragment of keratin 19). Green spots show a 2 fold increase upon removal of estradiol (eg Keratin 8) while yellow colored spots represent proteins decreased by the removal of estradiol (eg. Karyopherin, lamin).

Condition	Number of Unique Protein Features in Averaged Gels
MCF7 + E2	930
MCF7 - E2	814
LCC1 +E2	899
LCC1 -E2	891

Table 1. Summary of protein features comprising averaged gels for comparison across conditions. While 1200 to 1600 protein features were detected in each individual gel for each condition, these numbers represent protein features detected and matched in at least 4 out of every 6 gels for each condition.

Comparison	Increased	Decreased	Total change
MCF7 +E2 vs MCF7 - E2	45	29	74
LCC1 +E2 vs LCC1 - E2	13	47	60
LCC1 -E2 vs MCF7 - E2	73	51	124

Table 2. Effect of estradiol on MCF7 cells and on LCC1 cells. Shown are number of proteins showing greater than 4-fold changes in pH 5-8 2D gels.

Comparison	Increased	Decreased	Total change
MCF7 +E2 vs MCF7 - E2	44	26	70
Changes in common	1	3	4
LCC1 +E2 vs LCC1 - E2	12	44	56

Table 3. Effect of estradiol on MCF7 cells and on LCC1 cells. Shown are number of proteins with greater than 4-fold changes in pH 5-8 2D gels. The middle row shows those changes held in common between the two comparisons.

Comparison	Increased	Decreased	Total change
MCF7 +E2 vs MCF7 - E2	23	15	38
Changes in common	22	14	36
LCC1 -E2 vs MCF7 -E2	51	37	88

Table 4. Effect of estradiol on MCF7 cells and effect of acquired estradiol independence of LCC1 cells. Shown are number of proteins with greater than 4-fold changes in pH 5-8 2D gels. The middle row shows those changes held in common between the two comparisons.

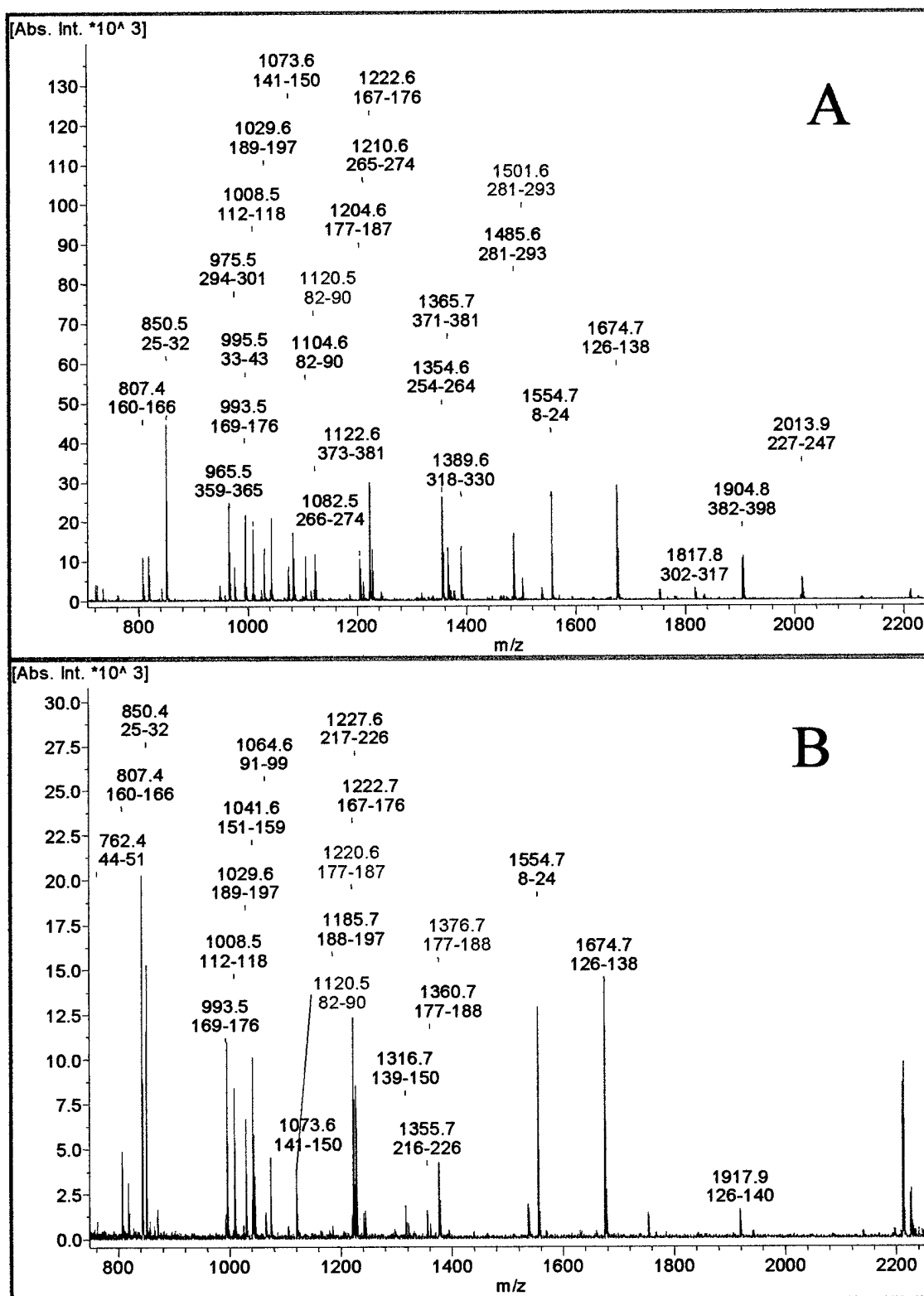


Figure 5. Tryptic peptide mass maps of full length (A) and proteolytically processed (B) keratin 19. The 2D gel spot giving rise to the processed form is only seen in MCF7 cells in the absence of estradiol (Figure 4).

Match to: **KRHU9**; Score: **338**

keratin 19, type I, cytoskeletal - human

Nominal mass (M_r): **44065**; Calculated pI value: **5.04**

A

Fixed modifications: Carbamidomethyl (C)

Variable modifications: Oxidation (M)

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P

Number of mass values searched: **35**

Number of mass values matched: **26**

Sequence Coverage: **59%**

Matched peptides shown in Bold Red

1 MTSYSYRQSS ATSSFGLGG GSVRFPGVA FRAPSIHGGG GGRGVSVSSA
 51 RFVSSSSSGG YGGGYGGVLT ASDGLLAGNE KLTMQNLNDR LASYLDKVR
 101 LEAANGELEV KIRDWYQKQG PGPSRDYSHY YTTIQDLRDK ILGATIENSR
 151 IVLQIDNARL AADDERTKFE TEQALRMSVE ADINGLRRVL DELTLARTDL
 201 EMQIEGLKEE LAYLKKNHEE EISTLRGQVG GQVSVEVDSA PGTDLAKILS
 251 DMRSQYEVMA EQNRKDAEAW FTSRTEELNR EVAGHTEQLQ MSRSEVTDLR
 301 RTLQGLEIEL QSQLSMKAAL EDTLAETEAR FGAQLAHIOA LISGIEAQLG
 351 DVRADSERQN QEYQRLMDIK SRLEQEIATY RSLLEGQEDH YNNLSASKVL
 401

Match to: **KRHU9**; Score: **251**

keratin 19, type I, cytoskeletal - human

Nominal mass (M_r): **44065**; Calculated pI value: **5.04**

B

Fixed modifications: Carbamidomethyl (C)

Variable modifications: Oxidation (M)

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P

Number of mass values searched: **46**

Number of mass values matched: **23**

Sequence Coverage: **44%**

Matched peptides shown in Bold Red

1 MTSYSYRQSS ATSSFGLGG GSVRFPGVA FRAPSIHGGG GGRGVSVSSA
 51 RFVSSSSSGG YGGGYGGVLT ASDGLLAGNE KLTMQNLNDR LASYLDKVR
 101 LEAANGELEV KIRDWYQKQG PGPSRDYSHY YTTIQDLRDK ILGATIENSR
 151 IVLQIDNARL AADDERTKFE TEQALRMSVE ADINGLRRVL DELTLARTDL
 201 EMQIEGLKEE LAYLKKNHEE EISTLRGQVG GQVSVEVDSA PGTDLAKILS
 251 DMRSQYEVMA EQNRKDAEAW FTSRTEELNR EVAGHTEQLQ MSRSEVTDLR
 301 RTLQGLEIEL QSQLSMKAAL EDTLAETEAR FGAQLAHIOA LISGIEAQLG
 351 DVRADSERQN QEYQRLMDIK SRLEQEIATY RSLLEGQEDH YNNLSASKVL
 401

Caspase consensus cleavage site: [ILV]ExD, shown in Bold Blue

Figure 6. Sequence coverage of full length (A) and proteolytically processed (B) keratin 19 corresponding to the peptide mass maps shown in Figure 5. The experimental pI and mass values match the calculated values in A but not in B.

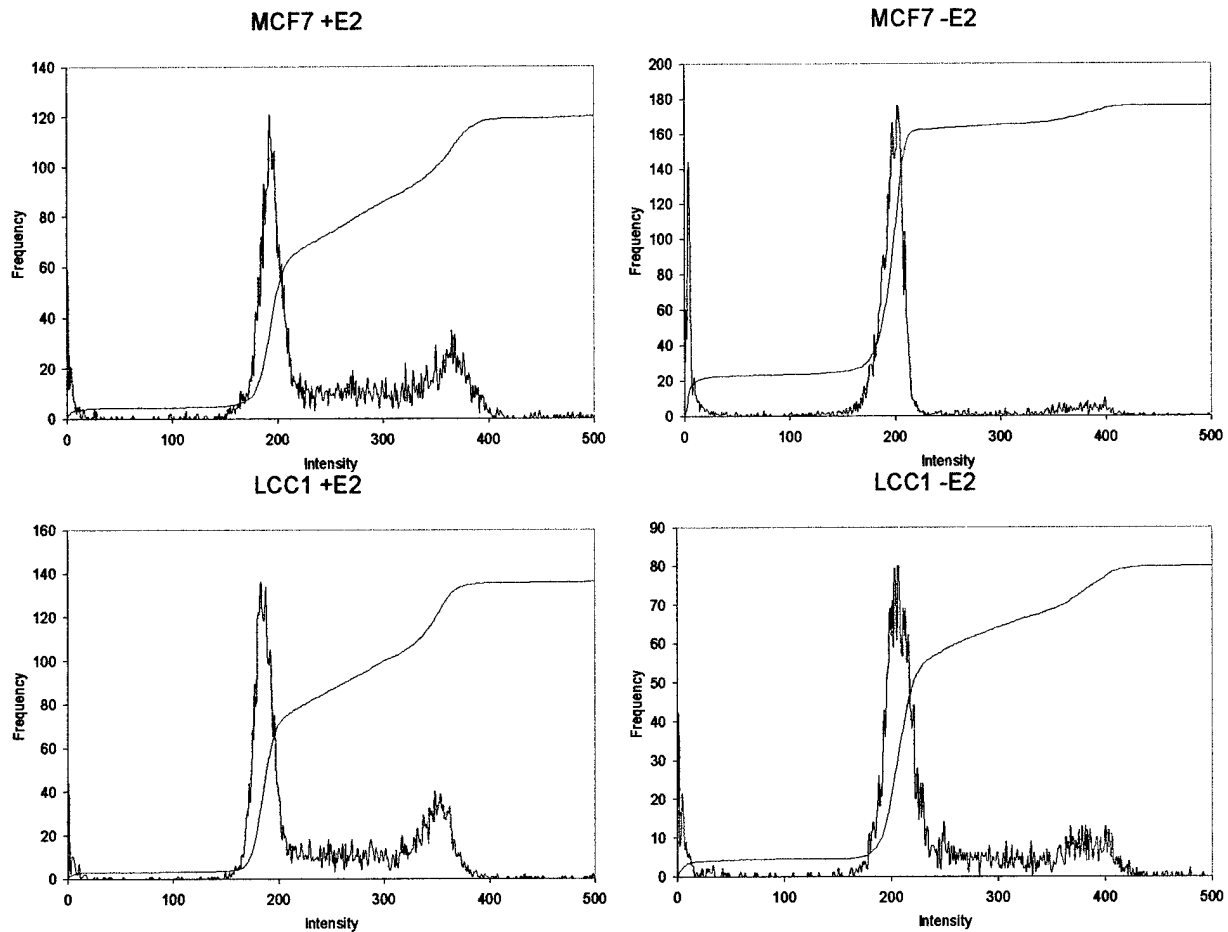


Figure 7. Effect of E_2 on mitotic index of MCF7 and LCC1 cells. Flow cytometry was used to measure DNA content of propidium iodide stained cells.

FileName	Status	Score	Protein MW	Title	Coverage
MCF7-E2 vs mCF7+E2 = MCF7-E2 vs LCC1-E2					
1 D:\BrukerNewData\0204\SOS\SOS011SF	Identified (multipl	93	94001.87	HSU46838 NID: - Homo sapiens	13 62
2 D:\BrukerNewData\0204\SOS\SOS021SF	Identified (multipl	184	94001.87	HSU46838 NID: - Homo sapiens	22 61
3 D:\BrukerNewData\0204\SOS\SOS031SF	Identified (multipl	117	86417.85	CDNA FLJ12466 FIS, CLONE NT2RM1000826, HIGHLY SIM	19 60
4 D:\BrukerNewData\0204\SOS\SOS041R	Undefined	77	100692.93	alpha-catenin 1 - human	11 59
5 D:\BrukerNewData\0204\SOS\SOS05b11	Identified (multipl	365	81856.93	replication licensing factor MCM7 - human	53 58
5 D:\BrukerNewData\0204\SOS\SOS05b11	Identified (multipl	400	81856.93	replication licensing factor MCM7 - human	61 57
6 D:\BrukerNewData\0204\SOS\SOS06b11	Identified (multipl	108	59720.01	keratin 10, type I, cytoskeletal - human	23 56
6 D:\BrukerNewData\0204\SOS\SOS06a2	Identified (multipl	153	59720.01	keratin 10, type I, cytoskeletal - human	33 55
7 D:\BrukerNewData\0204\SOS\SOS071SF	Identified (multipl	87	64938.57	HSU37436 NID: - Homo sapiens	22 54
8 D:\BrukerNewData\0204\SOS\SOS081SF	Identified (multipl	493	66521.75	LAMIN B1 - Homo sapiens (Human)	57 53
9 D:\BrukerNewData\0204\SOS\SOS091SF	Identified (multipl	154	58168.15	nuclear localization sequence receptor SRP1 alpha - human	48 52
10 D:\BrukerNewData\0204\SOS\SOS101SF	Identified (multipl	137	46751	CDNA FLJ20570 FIS, CLONE REC00956 (FRAGMENT) - Ho	40 51
11 D:\BrukerNewData\0204\SOS\SOS111R	Identified (multipl	109	59720.01	keratin 10, type I, cytoskeletal - human	28 50
12 D:\BrukerNewData\0204\SOS\SOS121S	Identified (multipl	240	51087.15	HSY13286 NID: - Homo sapiens	57 49
13 D:\BrukerNewData\0204\SOS\SOS131R	Identified (multipl	83	46979.26	ribonucleoprotein La - human	25 48
14 D:\BrukerNewData\0204\SOS\SOS141R	Identified (multipl	180	38624.27	MEMBRANE ASSOCIATED PROTEIN SLP-2 (STOMATIN-LI	47 47
15 D:\BrukerNewData\0204\SOS\SOS151S	Undefined	61	47890.31	DJ657E11.4 (SIMILAR TO 60S ACIDIC RIBOSOMAL PROTEI	22 46
16 D:\BrukerNewData\0204\SOS\SOS161S	Identified (multipl	118	37946.43	arapxin 2 - human	36 45
17 D:\BrukerNewData\0204\SOS\SOS171S	Identified (multipl	73	36383.56	4921530D09RIK PROTEIN - Mus musculus (Mouse)	30 44
18 D:\BrukerNewData\0204\SOS\SOS181S	Identified (multipl	184	30683.15	UNKNOWN (PROTEIN FOR MGC:10739) (SIMILAR TO HETE	48 43
19 D:\BrukerNewData\0204\SOS\SOS191S	Identified (multipl	154	34991.79	AF000576 NID: - Rattus norvegicus	47 42
20 D:\BrukerNewData\0204\SOS\SOS201S	Identified (multipl	126	40820.89	CGI-52 PROTEIN - Homo sapiens (Human)	39 41
21 D:\BrukerNewData\0204\SOS\SOS211S	Identified (multipl	180	38757.35	HUMPOLACCA NID: - Homo sapiens	56 40
22 D:\BrukerNewData\0204\SOS\SOS221S	Undefined	64	28850.02	HUMRPS6A NID: - Homo sapiens	26 39
23 D:\BrukerNewData\0204\SOS\SOS232S	Identified (multipl	214	33835.97	thiosulfate sulfurtransferase (EC 2.8.1.1) - human	52 38
24 D:\BrukerNewData\0204\SOS\SOS242R	Identified (multipl	128	17688.21	HSBT3 NID: - Homo sapiens	62 37
25 D:\BrukerNewData\0204\SOS\SOS251S	Identified (multipl	112	17808.03	dUTP pyrophosphatase (EC 3.6.1.23) - human	65 36
26 D:\BrukerNewData\0204\SOS\SOS261R	Identified (multipl	90	21541.68	HYPOTHETICAL 21.5 KDA PROTEIN - Homo sapiens (Huma	51 35
27 D:\BrukerNewData\0204\SOS\SOS271S	Undefined	62	19910.86	modifier protein 2 - mouse	31 34
28 D:\BrukerNewData\0204\SOS\SOS281S	Identified (multipl	153	26457.54	cathepsin d (EC 3.4.23.9), chain B - human	56 33
29 D:\BrukerNewData\0204\SOS\SOS291S	Identified (multipl	320	92686.51	endoplasmic precursor - human	36 32
30 D:\BrukerNewData\0204\SOS\SOS301R	Identified (multipl	264	61187.48	chaperonin GroEL precursor - human	51 31
31 D:\BrukerNewData\0204\SOS\SOS311S	Identified (multipl	255	50804.01	tubulin alpha-1 chain - Chinese hamster	63 30
32 D:\BrukerNewData\0204\SOS\SOS321S	Identified (multipl	174	56318.48	ATP SYNTHASE BETA CHAIN, MITOCHONDRIAL PRECUR	48 29
33 D:\BrukerNewData\0204\SOS\SOS331S	Identified (multipl	271	50095.24	tubulin beta-7 chain - chicken	89 28
34 D:\BrukerNewData\0204\SOS\SOS341S	Identified (multipl	304	36243.5	EUKARYOTIC TRANSLATION INITIATION FACTOR 2 ALPHA	67 27
35 D:\BrukerNewData\0204\SOS\SOS352S	Identified (multipl	110	36031.42	UNKNOWN (PROTEIN FOR MGC:4272) - Homo sapiens (Hu	32 26
36 D:\BrukerNewData\0204\SOS\SOS361S	Identified (multipl	265	47305.2	HSCYK18 NID: - Homo sapiens	39 25
37 D:\BrukerNewData\0204\SOS\SOS371R	Identified (multipl	269	42386.04	MMEIF4A NID: - Mus musculus	53 24
Increased in -E2					
1 D:\BrukerNewData\0204\SOS\SOS382S	Identified (multipl	144	51229.57	dC stretch-binding protein CSBP - rat	39 23
2 D:\BrukerNewData\0204\SOS\SOS391S	Identified (multipl	141	51229.57	dC stretch-binding protein CSBP - rat	33 22
3 D:\BrukerNewData\0204\SOS\SOS401S	Identified (multipl	127	106126.31	SEQUENCE 5 FROM PATENT WO9724448 - unidentified	19 21
4 D:\BrukerNewData\0204\SOS\SOS411S	Identified (multipl	125	74539.76	KAA1499 PROTEIN (FRAGMENT) - Homo sapiens (human)	28 20
5 D:\BrukerNewData\0204\SOS\SOS421S	Identified (multipl	157	59720.01	keratin 10, type I, cytoskeletal - human	34 19
6 D:\BrukerNewData\0204\SOS\SOS431S	Identified (multipl	531	65152.62	lamin C - human	66 18
7 D:\BrukerNewData\0204\SOS\SOS441S	Identified (multipl	444	74378.88	lamin A - human	54 17
8 D:\BrukerNewData\0204\SOS\SOS451S	Identified (multipl	106	56744.26	catalase (EC 1.11.1.6), chain D - human	31 16
9 D:\BrukerNewData\0204\SOS\SOS461S	Identified (multipl	107	55673.65	HSA007702 NID: - Homo sapiens	32 15
10 D:\BrukerNewData\0204\SOS\SOS471R	Identified (multipl	186	53529.03	HUMDKERB NID: - Homo sapiens	29 14
11 D:\BrukerNewData\0204\SOS\SOS481S	Identified (multipl	412	53529.03	HUMDKERB NID: - Homo sapiens	62 13
12 D:\BrukerNewData\0204\SOS\SOS492R	Identified (multipl	335	53529.03	HUMDKERB NID: - Homo sapiens	59 12
13 D:\BrukerNewData\0204\SOS\SOS501R	Identified (multipl	497	53529.03	HUMDKERB NID: - Homo sapiens	63 11
14 D:\BrukerNewData\0204\SOS\SOS511S	Identified (multipl	251	37849.68	HSA9985 NID: - Homo sapiens	66 10
15 D:\BrukerNewData\0204\SOS\SOS521S	Undefined	64	33746	T-CELL RECEPTOR ALPHA CHAIN - Mus musculus (Mouse	24 9
16 D:\BrukerNewData\0204\SOS\SOS531S	Identified (multipl	223	36392.72	annexin III - human	54 8
17 D:\BrukerNewData\0204\SOS\SOS541R	Identified (multipl	149	37687.56	HSTALDR3 NID: - Homo sapiens	34 7
18 D:\BrukerNewData\0204\SOS\SOS551S	Identified (multipl	165	28468.83	calpain (EC 3.4.22.17) small chain - human	42 6

Table 5. Partial list of E₂ regulated proteins in MCF7 cells and proteins constitutively regulated in LCC1 cells. The upper and lower groups represent proteins upregulated or downregulated, respectively, by E₂ in MCF7 cells or constitutively so regulated in LCC1 cells. Those entries shown in red in the sixth column are held in common when comparing the E₂ effect on MCF7 cells with the effect of phenotype switch between MCF7 and LCC1 cells.

LIST OF PERSONNEL

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